

Mix and Match: Transplanting symbiotic fungal partners across elevational gradients to gauge responses in migrating *Elymus* hosts

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Abstract

Within the next century, ecosystems are projected to experience climate warming, with strong effects in mountain systems. Warming can alter species distributions, with documented upward migrations along elevation gradients. Species interactions may be important factors that promote species establishment during climate-induced migration. For plants, fungal interactions could help or hamper establishment, but little research has addressed this issue in mountain ecosystems. As plants move up-slope, they may bring fungal symbionts with them, encounter novel fungal consortia, or have the roles of their symbionts change. To investigate fungal symbionts during upward migration, I performed a field study that transplanted a low-elevation restricted grass species (*Elymus elymoides*) and broadly distributed grass species (*E. trachycaulus*) into low- and high-elevation sites. At each site, I imposed three soil treatments: sterile soil, fungal spores added from low-elevation sites, or fungal spores added from high-elevation sites, to create mismatches between plants and fungi (e.g., low elevation fungal spores added in high elevation sites). I hypothesized that plant performance would be reduced when the symbiont was mismatched with its abiotic environment. After two years, *E. elymoides* transplanted into high-elevation sites had a reduced survival when inoculated with low-elevation fungi relative to high elevation fungi or sterile soil, suggesting that low elevation fungal communities were pathogenic at high elevation. Unexpectedly, a fungal mismatch increased growth for both species in low-elevation sites. These results demonstrate the potential for climate change to induce mismatches between plants and fungal symbionts that could affect their future fitness and geographic distributions.

Introduction

Current climate predictions for the 21st century predict patterns of higher mean annual temperatures leading to increased aridity coupled with of irregular precipitation and snow accumulation for much of North America (IPCC, 2014; Solomon, 2007). These changes in climate will have large implications for species in affected areas, likely altering their distributions as abiotic constraints on species ranges become more relaxed.

In mountain ecosystems, plants are predicted to move up in elevation as climate warms. The distributions of species that have been historically concentrated at low-elevations are predicted to rise and converge on high-elevation peaks, as modeled in mapping experiments in the Inyo and White

Mountains of California, U.S.A. (Van de Ven et al., 2007). Van de Ven et al. mapped current and predicted plant distributions under 3 °C warming to find changes in distribution – with species converging on cool, north-facing, high elevation slopes. Long-term observational studies at the Rocky Mountain Biological Lab (RMBL) in Colorado, U.S.A have suggested that the upward migration of several species of graminoids up alpine slopes has also been facilitated by warming (Zorio et al., 2016). This idea of upward migration of plant communities has been coined as the “mountain-top squeeze scenario” (Van de Ven et al., 2007), where low-elevation plants will invade the ranges of high elevation plants, assisted by warming temperatures expanding their physical ranges. Another study conducted at RMBL has shown that 20 years of experimental warming compared to un-warmed controls, had shifted the composition of a sub-alpine meadow to be predominantly composed of sedges as compared to its original composition of grasses (Rudgers et al., 2014).

The success of establishment by low-elevation species into novel high-elevation areas is dependent on a multitude of factors, including species interactions, which will be the focus of this research. In addition to navigating a novel environment of abiotic conditions, species that expand their ranges upward will also be faced with new biotic challenges, such as interactions with new competitors, pathogens, and potential symbionts, or losing such key interactions.

While several studies have focused on how population demographics and community compositions are affected by climate change, the literature investigating how species interactions are affected is underdeveloped. Many studies investigating effects on species interactions focus on how plant-pollinator interactions are affected by changes in plant phenology (Tylianakis et al., 2008; Morton and Rafferty, 2017), with even fewer studies investigating effects on interactions between plants and other interactors.

Plant-microbe interactions are of great importance to plant performance – particularly important are their mutualisms with belowground fungal partners. 80% of plants are colonized by a belowground fungal partner (Smith and Read, 2008), usually Arbuscular Mycorrhizal Fungi (AMF) or Dark Septate Endophytes (DSE). The compositions and distributions of belowground fungal groups have been found to change with elevation along gradients (Kivlin et al., 2017), and it is suggested that they may change with climate change (Giauque and Hawkes, 2016). On their own, these fungal symbionts serve a range of ecosystem services that include carbon sequestration, decomposition, and contributing to nutrient cycling in terrestrial systems (Langley and Hungate, 2003). Fungal symbionts aid their plant

hosts with nutrient acquisition and carbon sequestration, and also buffer their hosts against the detrimental effects of low water availability (Kivlin et al., 2013).

Graminoids are major players in global and local carbon and nutrient cycles and play a role as foundation species in montane ecosystems (Shantz, 1954). Their mutualisms with belowground fungi are expected to either assist or hinder them as the climate in montane slopes is predicted to become more arid and warm. However, it is unknown how climate change will affect the mutualism between graminoids and fungi – specifically when graminoids undergo upward migration, which may jumble the partnerships between specific mutualists. As graminoids move up-slope, they may lose their associated fungal partners, co-disperse with their symbionts, or be introduced to a novel environment of fungal consortia. In addition, the roles of some mutualistic fungal partners may change with elevation, having either a beneficial, antagonistic, or commensal effect on its host plant that has yet to be investigated in natural systems.

In this research, I asked if mismatches with fungal symbionts can affect host plants that are undergoing range shifts due to climate change in montane systems. Answering this question requires inquiry about differences in mutualisms at different elevations as graminoids are expected to move up-slope according to the mountain-top squeeze scenario. How will host plants and their symbionts be affected when they move into a new environment with potentially drastically different abiotic and biotic conditions?

I conducted a field experiment along 3 independent elevational gradients on slopes in mountain ecosystems to test how host plant performance is affected by upward migration and changes in species interactions with mismatched fungal consortia. I hypothesized that host plants would experience reduced performance when they were mismatched with fungal symbionts in a novel abiotic environment.

Methods

Field Methods

Three independent elevational gradients corresponding to separate watersheds – the Slate River Valley, Washington Gulch, and the East River Valley, in Gunnison County, Colorado are the locations of my sites (Fig. 1). Within each elevational gradient, there is one high-elevation site and one low-elevation site. The high elevation sites are ~430 m above the low elevation sites in the same area. These sites are

defined by the distributional patterns of 2 host species distributions. 2 species of the genus *Elymus* within the Poaceae sub-tribe Triticeae were used in the experiment. *Elymus elymoides* has a restricted low-elevation distribution, so the low-elevation sites are within its range in each gradient area. *Elymus trachycaulus* has a broader distribution that overlaps with that of *E. elymoides*, and the high elevation sites are in areas of the elevational gradient where the two distributions do not overlap.

At each site, there are 9 – 1 m x 2 m plots with 6 individual pots each (Fig. 2). Each pot contains an *Elymus* individual growing in a fungal exclusion cylinder (Fig. 3, 15 cm deep, 0.45µm nylon mesh bag secured to 5 cm diameter x 3 cm deep PVC ring), which will prevent belowground fungi from growing into or out of the pot, but will allow normal water and nutrient flow (Allison et al., 2013). Pots have received one of three soil treatments: sterile soil, soil from high-elevations that contains fungi from that area, and soil from low-elevations containing fungi from those areas. Sites have been maintained and monitored for 2 years.

To gauge host responses, measurements of the largest individual in each pot were taken every two weeks. Each week, a census of live plant number, dead plant number, and number of other species was also recorded. For the largest individual in each pot, various response variables including height (cm), tiller number, live leaf number, dead leaf number, and damaged leaf number were measured every other week. To reduce competition with other species and contamination by external fungi, forbs and other seedlings that grow into and around the pots were removed and collected. The summer of this experiment's duration was exceptionally dry, so to avoid confounding effects, each pot at each site was watered with ~35 ml of UltraPure milliQ water once a week. In addition, to ensure that neither focal species established into the area surrounding the sites, immature florets were removed and collected before pollen could be dispersed. This survey spanned 9 weeks during the summer of 2018.

Statistical Methods

I used general linear mixed effects models including the fixed effects of species, elevation (high/low) and fungal treatment (high/low/none), all possible interactions among the fixed effects, and the random effects of gradient (East River, Washington Gulch, Slate River), site (nested within gradient) and block (nested within site). I used model selection procedures based on AICc criterion to determine the best combination of random effects for each response variable. I used the <lme4> package for continuous response variables (tiller number, which is highly correlated with biomass) and <glmer> for the 0/1 response of survival. Tiller number was square-root transformed to meet assumptions of

normality of residuals and homogeneity of variances. To decompose species X fungal treatment X elevation interactions, I conducted planned, pairwise contrasts between fungal treatments for each species and elevation combination using the package <emmeans>. All analyses were conducted in R (R Core Team 2018).

Results

Plant Survival

Although it was not statistically significant, there was a trend towards higher proportional survival for *E. elymoides* at high elevations when matched with high-elevation fungi (Fig. 4, $P = 0.08$). Fungal treatments had no effects on survival of *E. elymoides* at the low elevation sites (Fig. 4). *E. trachycaulus* also had no significant differences among fungal treatments in the proportion of plants that survived at high or low elevation sites (Fig. 5).

Plant Growth

Tiller number was used as a proxy for measuring growth. At low elevations, *E. elymoides* had increased growth when given a high or sterile fungal treatment compared to ambient, low-elevation fungi (Fig. 6). At low elevations, *E. trachycaulus* had higher growth when given high elevation fungi, despite an environmental mismatch (Fig. 7). At high elevations, there were no significant differences between fungal treatments for either species (Fig. 6-7)

Discussion

The results of this study supported our hypothesis that plant performance would be reduced with a mismatched symbiont. While we found transplanting mismatched fungal partners to be beneficial for growth at low elevations for both of our study species, host survival was still negatively affected by mismatches in symbiont and environment (Fig. 4 – 7). High-elevation fungi treatments excelled in increasing tiller number of hosts at low-elevations, implying that they may increase growth in warming climates, rather than cause declines.

Ranelli et al. (2015) suggest that colonization by fungal partners and their response to climate change is context-dependent on abiotic and biotic conditions including host identity, elevation, and soil conditions. Climate change may change the outcomes of plant-fungal interactions. Our inclusion of a sterile soil treatment will allow us to determine if the fungal partner was mutualistic or pathogenic.

Future directions for this research would include analyses on biomass data, and sequencing cylinder soil from before and after the duration of the transplant experiment for unique fungal OTUs (Operational Taxonomic Units). Sequencing for fungal OTUs will inform us of the dominant fungal groups at each site, and changes in symbiont compositions over 2 years.

Conclusions

After 2 years of monitoring, our findings suggest that the upward migration of low elevation restricted species like *E. elymoides* may be facilitated by high elevation-fungi, and that high-elevation fungi may be able to withstand warmer conditions. The reasons for this may include a change in interaction outcome based on elevation or temperature or some other abiotic or biotic stressor.

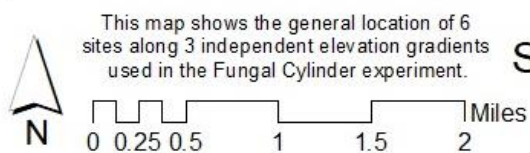
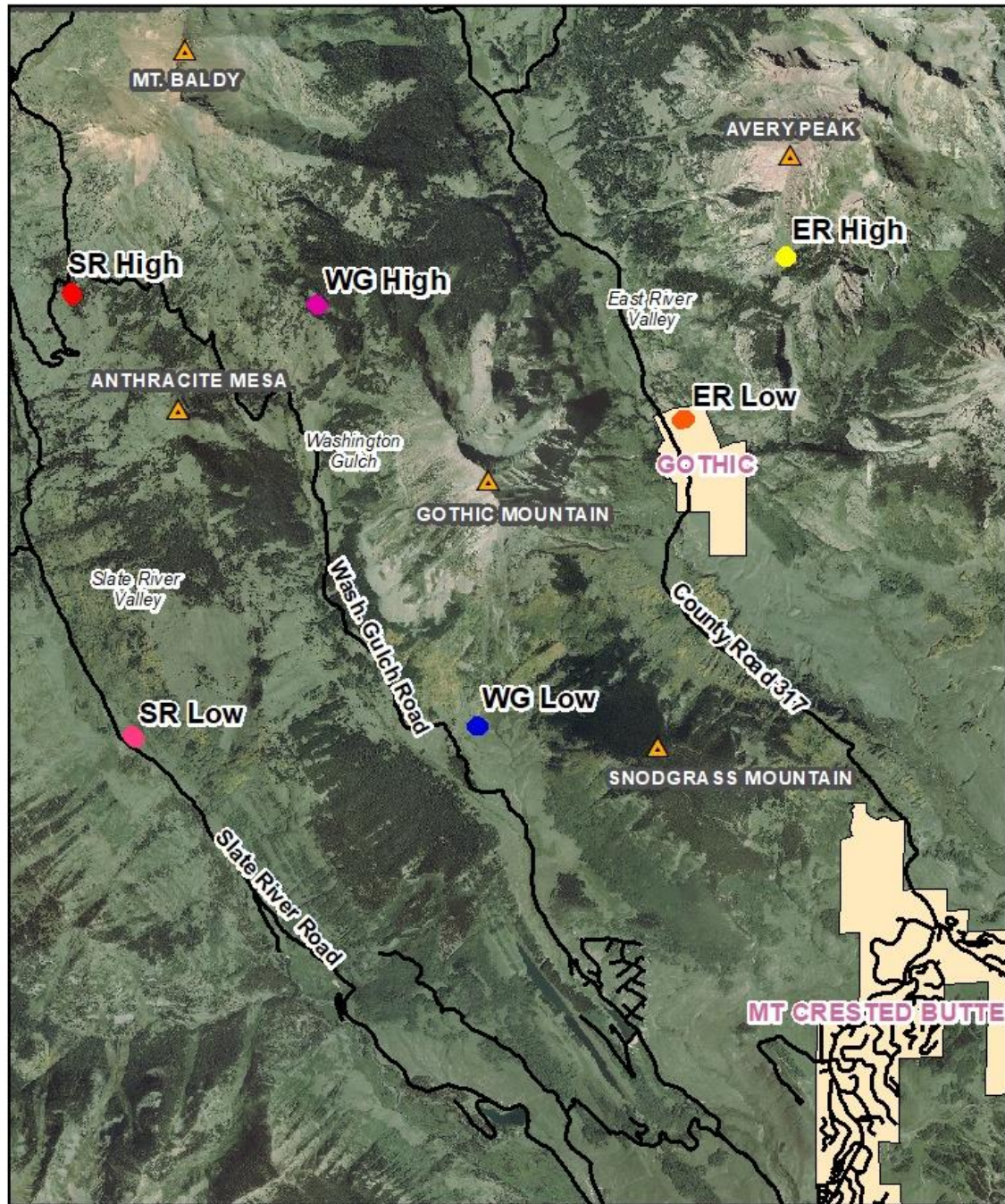
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Figures

Fig. 1 – Vicinity Map. This map shows the general location of 6 sites, 3 for each elevational gradient.



Sites in Gunnison County, CO.

Made by Kat Anderson, July 26, 2018

Fig. 2 – Plot Maps – This map shows the perimeter of the 9 plots within each site.

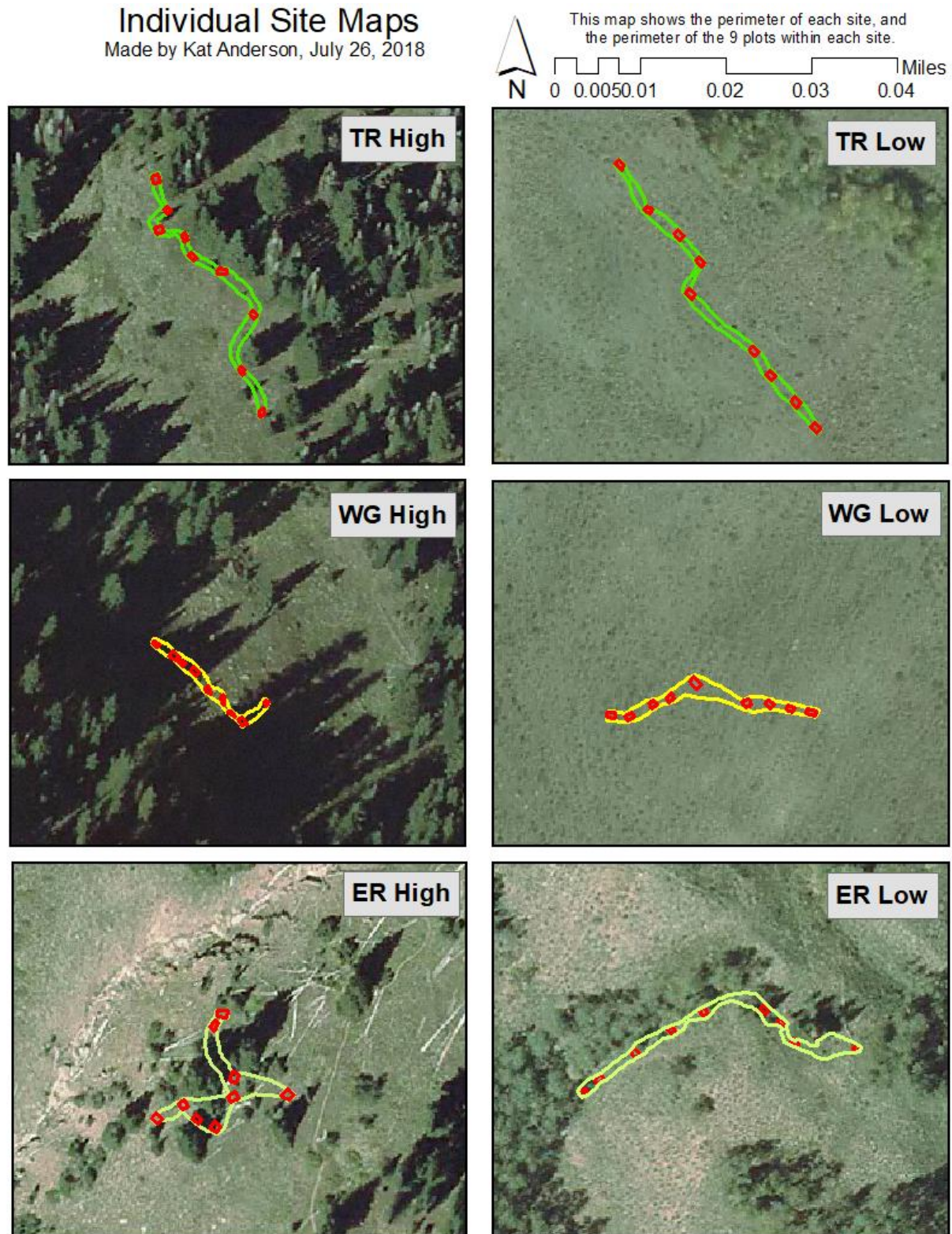


Fig. 3 – A fungal exclusion cylinder (15 cm deep, 0.45µm nylon mesh bag secured to 5 cm diameter × 3 cm deep PVC ring).



Fig. 4 – Proportional plant survival of *E. elymoides* at low and high elevation sites ($P = 0.08$).

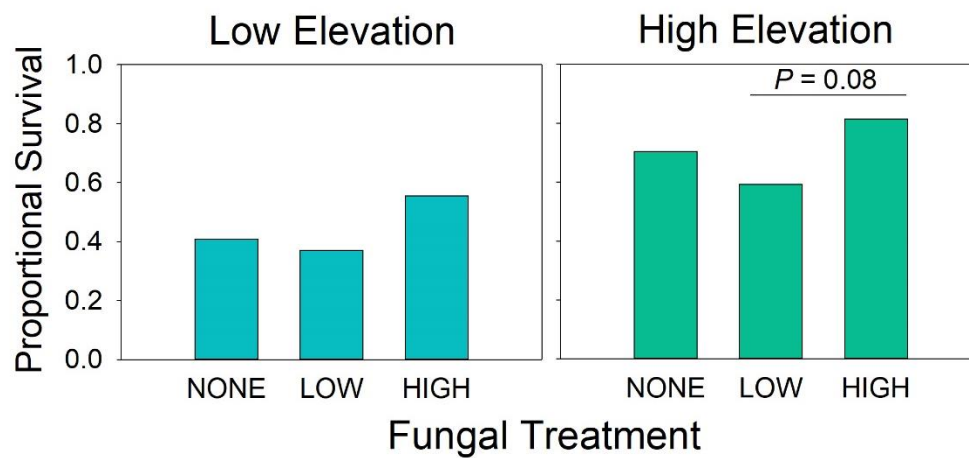


Fig. 5 – Proportional plant survival of *E. tracycaulus* at low and high elevation sites. No significant results.

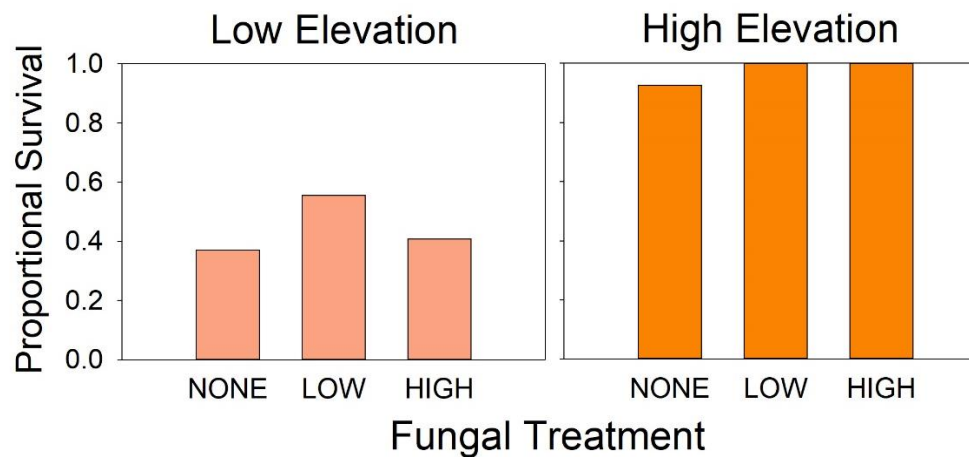


Fig. 6 – Mean tiller number, representing growth, of *E. elymoides* at low and high elevation sites. Significance denoted by differences in letters above treatment bars.

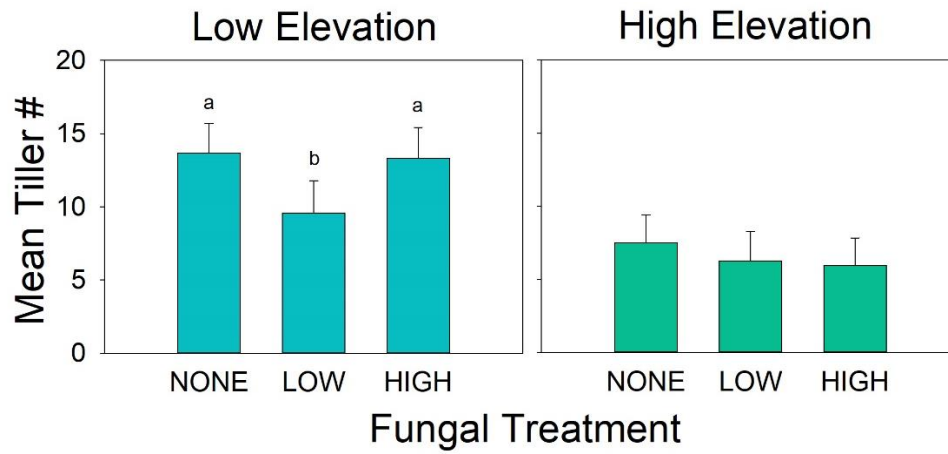


Fig. 7 – Mean tiller number, representing growth, of *E. trachycaulus* at low and high elevation sites. Significance denoted by differences in letters above treatment bars.

